FIELD AND LABORATORY STUDIES OF VERBENA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 257

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(WITH PLATES VI–IX AND TWENTY-SIX FIGURES)

Introduction

In GRAY'S *New Manual of Botany* (edition of 1908), 8 species of *Verbena* are described as occurring in the Eastern United States. These are classified into two sections, of which the first is further subdivided into three groups. Five of the 8 species grow wild in the vicinity of Chicago, namely, *Verbena urticaefolia* L. and *V. bracteosa* Michx., belonging to the first and third groups respectively, and *V. angustifolia* Michx., *V. hastata* L., and *V. stricta* Vent. to the second group. These three last named species occur abundantly at Stony Island, a southern suburb of Chicago, where the conditions of prairie, damp, and dry ground are met with successively as one proceeds from the north to the south end of the locality. Here the three forms grow in their characteristic ecological situations: *V. stricta* on the prairie, *V. hastata* in damp low places, and *V. angustifolia* on high dry ground. On examining the *Verbena* plants, one is rather surprised to find that there are many intermediate forms which can scarcely be assigned to any of the three species with certainty. The question arises, therefore, as to whether they are hybrids or mutants of the three species.

The present work was undertaken to determine whether or not there are any cytological differences in the fertilization phenomena and early stages of development between these forms. The results were rather negative as regards the genetic nature of the intermediate forms; that is, with slight exceptions, no significant differences were found between them. Many of the observations upon the embryonic development, however, are sufficiently interesting to merit description. These will therefore constitute the chief subject matter of the present paper, such facts and suggestions as I am able to present regarding the origin and nature of the intermediate forms being added at the close.

This work was carried on at the Hull Botanical Laboratory, University of Chicago, under Professor CHARLES J. CHAMBERLAIN, to whom I wish to express my sincere thanks for suggesting the problem, and my appreciation of his kind advice throughout the progress of the work. My acknowledgments are also due to Professor JOHN M. COULTER for his kindness in placing the conveniences of the laboratory at my disposal.

Taxonomic observations

Although one can easily recognize the specific characters of the original species, *V. angustifolia*, *V. stricta*, and *V. hastata*, it is impossible to arrange the forms intermediate between them in a linear series with regard to all of their contrasting characters. In other words, all of the characters do not vary in the same direction, so that if one distributes them among the original species with reference to one character, a different distribution would be required for some other character. Examples of the 3 species and the 6 intermediate forms which I was able to collect are given in figs. 1–9 (pl. VI). Figs. 1, 3, and 7 are *V. angustifolia*, *V. stricta*, and *V. hastata* respectively, and the others are the intermediates arranged between the 3 species in accordance with their degree of similarity to them, as nearly as this could be determined. I have attempted to represent diagrammatically the morphological relationship between the originals and the intermediates by the triangle shown in text fig. 10; the numbers on the triangle refer to the figures in plate VI. The three apices (1, 3, 7) indicate the three original species, and the points along the sides of the triangle show the probable position of the intermediate forms with reference to them. For example, 4 is believed to be nearer to 3 than to 7, and 5 is probably about midway between 3 and 7. The contrasting characters of all of the forms are given in detail in table I.

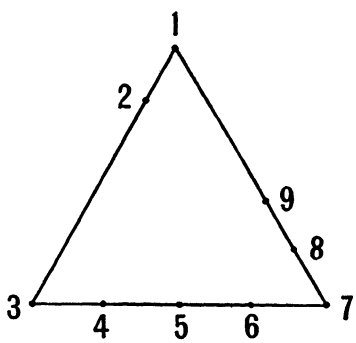


FIG. 10.—Diagrammatic representation of morphological relationship between originals and intermediates.

TABLE I

No.	Height in cm. in average	Stems			Leaves					Spikes		Corollas		Bracts
		Form of cross section	Branched or not	Stout (+) or slender (−)	Breadth in cm. in average	Length in cm. in average	Petiolated or sessile	Serrature double (+) or single (−)	Hair thick (+) or not (−)	Thick (+) or filiform (−)	Clustered (+) or loose (−)	Color	Size	Long (+) or short (−)
1...	25.2	Quadrangular	Apical part only	−	0.7	5.1	Sessile	−	− and rough	+ slightly	−	Pale purple	Medium	− and leafy
2...	30.0	Quadrangular	Apical part only	− but stronger than former	2.7	7.0	Sessile	−	+ and rough	+ than preceding	−	More violet than preceding	Slightly larger	+ and leafy
3...	88.0	Round	Not except sometimes at apical part	+	4.5	8.5	Sessile	+ heavy	+ and woolly	+ strongly	+	Purple	Large	+ and slightly leafy
4...	120.0	Round quadrangular	Not except sometimes at apical part	+	5.0	12.0	Short petioles	+ heavy	+ and slightly woolly	+	+	Purple	Large	−
5...	99.9	Round quadrangular	Not	+	3.2	9.0	Long petioles	+ slight	+ and rough	+	+ slightly	Lilac	Large	+ and narrow
6...	120.0	Quadrangular	Rare	+	2.7	8.5	Short petioles	+	+ rough and stiff	+ slightly	+ slightly	Purple	Slightly larger	−
7...	187.0	Quadrangular	Apical part only	+	2.5	11.4	Long petioles	+ slight	+ short	−	+	Purple lilac	Small	+ and narrow
8...	120.0	Quadrangular	Apical part only	Somewhat −	1.7	10.0	Long petioles	−	+ short	−	+ slightly	Pinkish purple	Slightly larger	−
9...	82.5	Quadrangular	Apical part only	Somewhat −	3.0	9.5	Long petioles	−	+ short	−	+	Pinkish purple	Slightly larger	−
1...	25.2	Quadrangular	Apical part only	−	0.7	5.1	Sessile	−	− and rough	+ slightly	−	Pale purple	Medium	− and leafy

It is necessary to consider whether or not the differences between these plants might not have been induced through adaptation and response to the local conditions in which each type may happen to be growing. Such an influence of local factors can be recognized at Stony Island in different degrees; thus, for instance, while the color of the flowers of *V. hastata* varies greatly with individuals, without reference to the conditions of the habitat, the shape and texture of the leaves of this species are plainly responsive to the surroundings, those plants growing in dry places having narrower and stiffer leaves than those inhabiting wet situations.

I believe I have eliminated this possibility in selecting my materials, and those which I regard as intermediate forms are not cases of modifications due to individual differences or adaptation to local conditions. Thus I have found forms 1 and 2 growing under the same external conditions at one location; forms 4, 5, and 6 growing together at another place; and forms 8 and 9 growing at a third spot.

Cytological observations

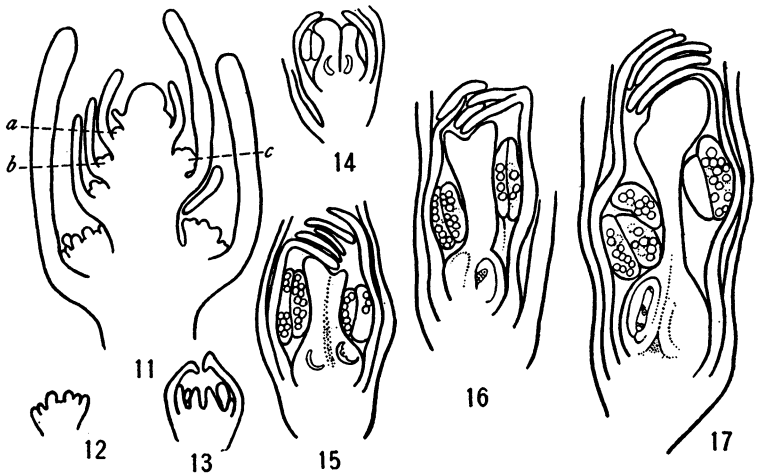
Material and methods

The spikes of *V. angustifolia* (fig. 1), *V. stricta* (fig. 3), and *V. hastata* (fig. 7), and the form intermediate between *stricta* and *hastata* (fig. 5) were collected during July and August 1918 at Stony Island. The apical part of the spikes, the pistils, and the young fruits in different stages of development were fixed in chrom-acetic acid and corrosive sublimate–acetic acid solutions, the former giving the best results. In the case of the pistils and fruits, it was found advantageous to pick off carefully or partially remove the calyx tubes, as they interfered with the rapid penetration of the fixing fluid. Sections of the apical part of the spikes were cut 5, 10, and 15 μ in thickness; pistils and young plants, 5 and 7.5 μ. Flemming's triple stain and iron alum haematoxylin were used, the former giving quite satisfactory results.

All of the four forms mentioned were examined in more or less complete series. *V. angustifolia* is chosen as a type for the purposes of description, but most of the statements are applicable to the others also, and they will be mentioned specifically only where differences between them make a separate discussion necessary.

DEVELOPMENT OF FLOWER

The first evidence of the formation of flowers is the appearance of papillae in the axils of the bracts (fig. 11*a*); these papillae are the primordia of the receptacles of the flowers. The outline of the receptacle soon becomes angular through the upward growth of four hemispherical protuberances from its distal surface (fig. 11*b*), and soon afterward its base produces a ring-shaped outgrowth (fig. 11*c*). The former develop into the stamens, and the ring immediately afterward separates into the corolla and the calyx



FIGS. 11–17.—Floral development in *V. angustifolia;* ×35

tube (fig. 12). The appearance of the carpels is indicated by a broadening of the receptacle (figs. 12, 13).

In fig. 13 the calyx tube has begun to curve inward over the top of the flower. Within this the corolla tube, the hemispherical young stamens and the two carpels appear in succession. Their later stages are shown in figs. 14–17.

DEVELOPMENT OF MEGASPORE AND EMBRYO SAC

When the ovule has reached the stage shown in fig. 15, the subepidermal megaspore mother cell that terminates the axial row of the nucellus can readily be distinguished from the surrounding cells through its larger size and large nucleus (fig. 18). The

megaspore mother cell and its nucleus with a prominent nucleolus continue to increase in size (fig. 19). Two divisions then occur which result in the typical formation of a row of four megaspores (figs. 20, 21); this takes place when the ovule is about at the stage represented in fig. 16. The innermost of the four megaspores is the largest, and is destined to develop into the embryo sac (fig. 22).

Successive stages in the development of this basal megaspore, accompanied by the destruction of the other three megaspores, are shown in figs. 22–25. The nucellus, consisting of a single layer of cells, surrounds the row of megaspores (fig. 21). It eventually becomes so distended by the enormous expansion of the developing embryo sac that it ruptures, and the ruptured nucellus is then carried downward as a cap on the growing embryo sac, as was previously described by MOTTIER (14) in *Arisaema*, CALDWELL (1) in *Lemna*, and MERRELL (13) in *Silphium*. In the next stage (fig. 26) the embryo sac lies free in the space between the funiculus and the integument, and the yellowish-brown remnants of the nucellus are observable capping the micropylar end of the sac.

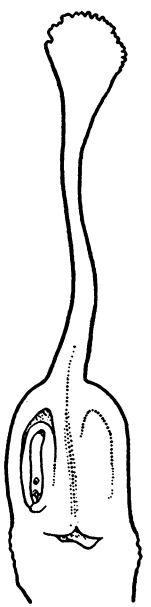


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FIG. 27.—*V. angustifolia:* mature pistil with mature embryo sac; ×35.

The phenomena of the enlargement of the sac, the division of its nuclei, and the destruction of the cells of the nucellus do not occur simultaneously, but these processes take place at different rates. The development of the megaspore and the fate of the nucellus are exactly the same as described by MERRELL for *Silphium*.

When the embryo sac reaches maturity (fig. 26), taken from an ovary in the stage represented in fig. 27, the sac is several times larger than it was when inclosed in the nucellus, very slender in shape, and always constricted just above the egg apparatus. The egg apparatus seems to be typical. The nucleus of the egg is several times larger than the nuclei of the synergids and contains

in the resting condition a fine chromatin network and a large, often vesicular, nucleolus. After the fusion of the polar nuclei, which occurs near the middle of the sac (fig. 25), the resulting endosperm nucleus approaches the egg apparatus. At this time, as shown in fig. 26, the endosperm nucleus still possesses two nucleoli, evidences of its binucleate origin, and is considerably larger than the egg nucleus. It is frequently in contact with the egg. There are three very small but typical antipodal cells.

The nutritive jacket surrounding the embryo sac of *Verbena* usually consists of a single layer of cells derived from the inner epidermal layer of the integument, and it develops especially at the micropylar end, investing the egg apparatus of the embryo sac. The cells of the jacket have conspicuous brownish contents, among which are numerous starch grains. Rather frequently a portion or portions of the jacket cells inclosing one or more grains of starch protrude into the embryo sac.

DEVELOPMENT OF MICROSPORES

At the stage shown in fig. 14 the hypodermal archesporial row is distinguishable, and the succeeding stages follow the usual course of development (figs. 28, 29). There may be only a single longitudinal row of spore mother cells, but one or two longitudinal divisions of the primary sporogenous row may take place (fig. 30).

The pollen mother cells within a loculus do not divide quite simultaneously, so that several different stages of the reduction division may be found among them (figs. 31–33). It is rather difficult to count the number of chromosomes in this species (*V. angustifolia*) because they are remarkably small and slender, but it was ascertained that 8 is the $2x$ number. In the second maturation division the two spindles usually lie across each other as in fig. 33.

In *V. angustifolia* there are two different types of tetrad formation. In the one case the peripheral cytoplasm of the pollen mother cell is left over to form a wall for the tetrad, this wall subsequently disintegrating (figs. 34, 35), while in the other case the entire mother cell is utilized in the formation of the tetrad (fig. 36). Figs. 37–41 give successive stages in the development of

the pollen grains. The wall of each microspore gradually thickens and sometimes a great many starch grains may be observed in the interior (fig. 39). Cases of accumulation of starch grains in the pollen have been reported by MURBECK (15), ISHIKAWA (11), and others. In *Oenothera* ISHIKAWA states that "the plasm containing starch grains in the pollen tube is poured into the attacked synergid," but in this case no starch is present in the pollen tube (fig. 42). A large vacuole appears in the pollen grain for a time (fig. 40), but it soon fades away and the first vegetative cell is cut off (fig. 41). More advanced stages could not be observed, as the contents and wall of the pollen grains become extremely dark in color. While these changes are occurring, the tapetum and middle layer disintegrate.

FERTILIZATION

It is very difficult to obtain clear pictures of the stages in which the male nuclei are on the point of fusing with the egg cell and the endosperm nucleus. In the first place the egg apparatus is rendered very indistinct through the presence of deeply staining cytoplasmic substances around it. I believe this deeply staining material is the result of a concentration of the cytoplasm and the inclusion within it of nutritive substances destined for the endosperm. The abundance especially of starch grains around the egg apparatus greatly confuses its appearance with the gentian violet stain. Secondly, the synergids seem to be more ephemeral in *Verbena* than in other plants, and soon become converted into a tenacious mucus-like material. This material from the disorganized synergids also stains very deeply. Thirdly, when the pollen tube enters the egg apparatus, a part of the disorganized nucellar cap penetrates into it with the tube and always gives rise to a figure of peculiar shape and staining properties (figs. 42–44, 46). MERRELL states that in *Silphium* "the pollen tube passes along the outside of the cap which usually crowns the embryo sac and enters the sac just beyond its free margin." In *Verbena*, however, the pollen tube, entering the sac at the micropylar end, thrusts itself through the nucellar cap (fig. 42), just as in *Lemna*, described by CALDWELL.

Figs. 43 and 44 show stages of fusion of the male and female nuclei. In fig. 43 one of the male nuclei is in contact with the egg and the other with the embryo sac nucleus, and in fig. 44 one of the male nuclei has fused with the egg nucleus.

In connection with the fertilization process it should be reported that at this time a proteid-like substance makes its appearance in the cavity between the carpels and ovules (figs. 26, 27). This material forms a network, probably as the result of coagulation by the fixing agent, and stains deeply with cytoplasmic dyes. The only suggestion which can be offered as to the function of this substance is that it may be related to the nutrition of the pollen tube, since it appears just before fertilization and disappears shortly after that process is completed.

Formation of endosperm

After fertilization the primary endosperm nucleus moves toward the center of the embryo sac, and its first division takes place there. This division is followed by the formation of a wall which divides the sac into two approximately equal chambers, the micropylar and the antipodal chambers (figs. 45, 46). Such a formation of a two-chambered embryo sac has been observed in many plants, both monocotyledons and dicotyledons, by Hofmeister (10), Schaffner (17), Campbell (2), Guignard (6), Hall (8), Murbeck (15), Cook (3), and others. Several other cases are mentioned by Coulter and Chamberlain (4).

The nucleus of the micropylar chamber gradually changes its position, moving toward the middle of the chamber, and soon afterward produces a great many free nuclei (figs. 46, 47), around which walls are subsequently formed, beginning at the micropylar end. This mode of development of the endosperm corresponds to the third type in Hegelmaier's (9) classification. Twelve chromosomes, that is, the $3x$ number, were often counted in these nuclear divisions. The nucleus of the antipodal chamber also moves toward the center of that chamber, and increases in size, but does not undergo division for a long time (figs. 46, 47). The antipodal chamber elongates like a haustorial tube, extending to the chalazal extremity of the ovule, sometimes becoming exceedingly curved.

Figs. 48 and 49 illustrate two parts of the same embryo sac; the endosperm tissue is seen to be fully formed in the micropylar chamber, while the antipodal chamber is still uninucleate.

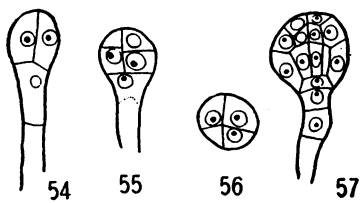
A large amount of starch is present in the embryo sac, as was also observed by GUIGNARD (7) (*Cestrum*), D'HUBERT (5) (Cactaceae), WEBB (18) (*Astilbe*), and LLOYD (12) (*Galicium*). This is observable not only a little before fertilization, but more especially after fertilization has occurred (figs. 43, 44, 46). Fig. 46 shows starch not only in the micropylar and antipodal chambers, but also even in the egg cell. It is evident that the starch grains in the micropylar chamber are always larger than those in the antipodal chamber. These starch grains are naturally closely related to those in the nutritive jacket. I have already mentioned that jacket cells loaded with starch grains may protrude into the sac. Sometimes one gains the impression that the starch grains have entered the sac through the destruction of the thin walls of the jacket cells. Such a direct transfer of starch, however, is hardly to be credited, partly because there are many fewer grains in the sac than in the jacket, but mainly because the walls of the jacket cells seem to be composed of very resistant material, since they persist for a long time apparently intact. In the *V. hastata* material I found occasionally an entire absence of starch grains in the jacket cells, and in such cases the development of the embryo sac is always remarkably retarded, and the egg apparatus is absent (fig. 50).

The further development of the endosperm is the same as in *Sagittaria*, described by SCHAFFNER (17). While the micropylar chamber is becoming filled with walled endosperm tissue through free nuclear division, the enlarged nucleus of the antipodal chamber still remains undivided. Sometimes it divides once or twice (fig. 51), forming two or three free nuclei which enlarge enormously. Meantime the endosperm tissue continues to develop, finally extending from the micropylar chamber into the antipodal chamber, forcing the large cell which occupies the antipodal chamber up to the antipodal end. At about this time the antipodal cells disintegrate (fig. 52). The large cell at the antipodal end of the chamber gradually diminishes in size, and finally disappears.

In COULTER and CHAMBERLAIN'S book (4) it is stated that "the endosperm is said to develop only in the antipodal chamber in *Loranthus*, Vacciniaceae, Verbenaceae, etc." This statement should be corrected as far as it concerns the various species of *Verbena* which I have studied.

DEVELOPMENT OF EMBRYO

The proembryo divides in two by a transverse wall and remains without further change for a long time (fig. 49). It then elongates, with accompanying divisions, reaching a condition like that illustrated in fig. 53, where it is a filament of varying length, consisting of several cells. The apical cell of the filament then divides longitudinally (fig. 54), followed by another longitudinal and a transverse division in either order, resulting in an octant stage (figs. 55, 56).



FIGS. 54–57.—*V. hastata:* successive stages of development of embryo; fig. 56, apical view of stage in fig. 55; ×400.

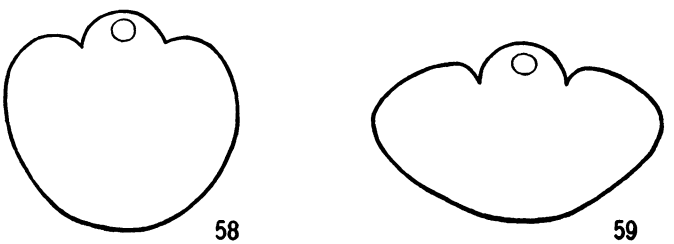
The dermatogen, periblem, and plerome layers are next differentiated in the embryo (fig. 57), which now occupies the end of a long suspensor. The appearance is identical with that of *Capsella*.

Relationship of intermediate forms

COOK, comparing two species of *Sagittaria*, *S. variabilis* and *S. lancifolia*, says: "With such striking external differences one would naturally expect equally interesting internal differences, but to my surprise I found the development of the embryo sac and embryo of *S. lancifolia* practically the same as had been described by SCHAFFNER for *S. variabilis*." I was equally surprised on comparing the forms of *Verbena*. I selected as the intermediate form for comparison with the original species the type designated in the earlier part of this paper as no. 5 (see fig. 5), because it is one of the most abundant of the intermediates and because it seemed to be halfway between *V. stricta* and *V. hastata*. In the following account the morphological and cytological characters of this intermediate are compared with those of the three species.

The flowering period of *V. angustifolia* comes earlier than that of *V. stricta*, *V. hastata*, and the intermediate form between them, so that the last three flower at the same time. For this reason one would expect that intermediate forms between *V. angustifolia* and the other two species would be rather rare, while those between *V. stricta* and *V. hastata* would be more common, if these intermediate forms are really hybrids. As a matter of fact, the relative abundance of the intermediates corresponded to the expectation.

The young ovule of *V. hastata* at the stage in which the megaspore mother cell first makes its appearance (fig. 15) is rounded (fig. 58), while that of the other three forms is somewhat flattened, as indicated in fig. 59. The young ovule of the intermediate form is therefore similar to that of *V. stricta*.



FIGS. 58, 59.—Diagrammatic outline of young ovule: fig. 58, *V. hastata;* fig. 59, other 3 forms.

The size of the mature embryo sac varies considerably within each species owing to individual variations, but an approximate comparison of its size at the same stage in the four forms can be made without difficulty. The following table gives the average length of 12 embryo sacs of the four forms at three different stages.

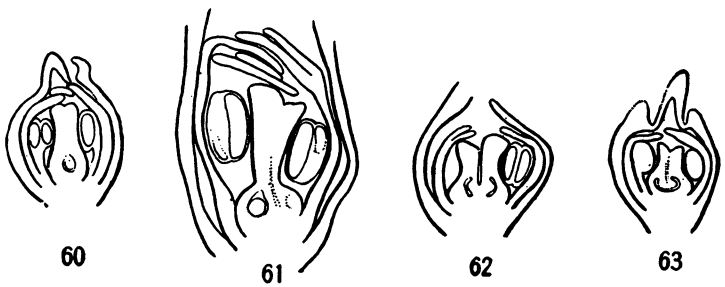
TABLE II

Name	*V. angustifolia*	*V. stricta*	Intermediate form between *V. stricta* and *V. hastata*	*V. hastata*
Fig. 26 stage.....	0.260 mm.	0.225 mm.	0.185 mm.	0.185 mm.
Fig. 51 stage.....	0.500	0.460	0.390	0.310
Fig. 56 or 57 stage	0.460	0.540	0.360	0.340

The breadth of the sac in all cases is about 0.02–0.03 mm. The figures show that with regard to the length of the embryo sac the intermediate form resembles *V. hastata* more than it does *V. stricta*.

At the time of the first mitosis of the microspore mother cell the flower buds of the 4 forms are in different stages of development. As shown in figs. 60–63, the buds of *V. angustifolia* and *V. hastata* are in a relatively young stage when this event occurs, those of *V. stricta* in a much later stage, and the intermediate form at a stage between these two. In respect to this character, then, the latter occupies an intermediate position.

As described in a preceding section, tetrad formation occurs in *V. angustifolia* in two different ways, with or without persistence of a rim of cytoplasm from the mother cell. In *V. stricta* the cytoplasm always persists in this manner, forming, even at the first mitosis of the microspore mother cell, a deeply stained border



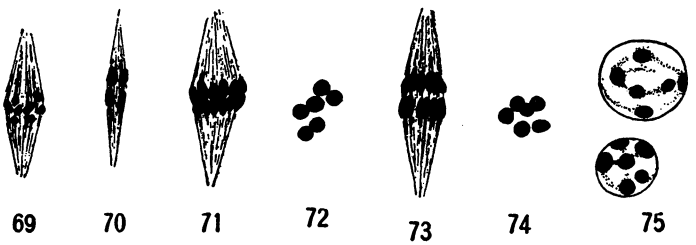
FIGS. 60–63.—Comparison of florets at time of first mitosis in pollen mother cells: fig. 60, *V. angustifolia;* fig. 61, *V. stricta;* fig. 62, intermediate form between *V. stricta* and *V. hastata;* fig. 63, *V. hastata;* ×35.

around the central portion where the mitosis is occurring (figs. 64, 65). In *V. hastata* no such cytoplasmic border is ever formed around the microspores, but all of the cytoplasm of the mother cell is utilized in the production of the pollen grains. The intermediate form is like *V. hastata* in this regard (figs. 66–68).

V. angustifolia has 8 chromosomes as the $2x$ number. A late prophase and metaphase of the first reduction division in this species are shown in profile view in figs. 69 and 70. The other 3 forms have 12 chromosomes as the $2x$ number. A metaphase of *V. stricta* and an early anaphase of the intermediate form from the side and end are illustrated in figs. 71–74. I regret that in *V. hastata* I was unable to find just the same stage to compare with these, as all of my material of this species is either a little too early or too

late. It is safe to conclude, however, that 12 is also the $2x$ number for this species, since in the early telophase of the first division (fig. 75) 6 chromosomes are clearly present at each pole of the spindle. I have further often counted 12 chromosomes in all of the forms except *V. angustifolia* in the anaphase stage in young locular cells of anthers, and 18 chromosomes, the $3x$ number, in the endosperm cells. The behavior of the chromosomes of the intermediate form in mitosis is entirely normal, and like that of the original species. No such abnormalities as were described by ROSENBERG (16) in *Drosera* hybrids can be recognized.

Owing therefore to the unfortunate fact, which could not be foreseen, that both of the original species selected for comparison with a form intermediate between them have the same number of



FIGS. 69–75.—Mitosis of pollen mother cell: figs. 69, 70, *V. angustifolia;* figs. 71, 72, *V. stricta;* figs. 73, 74, intermediate form between *V. stricta* and *V. hastata;* fig. 75, *V. hastata;* ×1500.

chromosomes, cytological observations upon them do not serve to settle the question as to whether the intermediate form is a hybrid or not. It is clear that the intermediate form does not differ cytologically from the original forms, and that its mitotic behavior is entirely normal. These facts, if they have any significance at all, tend to suggest that the intermediate is not a hybrid, but rather a mutant of one or the other of the original species. This could be determined only by breeding it through several generations and observing whether its characters are fixed or not.

Cytological studies of the forms intermediate between *V. angustifolia* and the other two species might have yielded more definite results, because it differs from them in the number of its chromosomes. Unfortunately I did not collect any material from these forms, as they are relatively rare.

Summary

Several intermediate forms were found between three species of *Verbena* which grow on Stony Island, *V. angustifolia* Michx., *V. stricta* Vent., and *V. hastata* L., which can be arranged taxonomically between the three species in question. Embryological and cytological studies were made on the three species and on one of the forms intermediate between *V. hastata* and *V. stricta* in order to determine the genetic nature of the intermediate.

From the cytological point of view, nucellar cap, nutritive jacket, and chambered embryo sac are pointed out as the characteristic features of these forms. The reduced number of chromosomes is 4 in *V. angustifolia* and 6 in the other three.

It was not possible to decide from the cytological studies whether the intermediate form is a hybrid or not, since both of the original species from which it might be supposed to have sprung were found to have the same number of chromosomes. The chromosome behavior of the intermediate was like that of the two species and entirely normal. Some of its developmental characters are intermediate and some are similar to either *V. stricta* or *V. hastata*.

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EXPLANATION OF PLATES VI–IX

Figs. 10–17, 27, 54–63, 69–75 are in the text; all the others in the plates. All drawings were made with an Abbé camera lucida at table level. Figs. 11–17, 27, and 60–63 were drawn with Zeiss compensating ocular no. 4 and Spencer 16 mm. objective; figs. 18–25, 28–41, and 64–68 with Reichert ocular no. 18 and Spencer 4 mm. objective; figs. 26 and 42–53 with Zeiss compensating ocular no. 4 and Bausch and Lomb 1/12 oil immersion objective; figs. 69–75 with Reichert ocular no. 18 and Bausch and Lomb 1/12 oil immersion objective. Text figures reduced one-half, plates nearly two-thirds in reproduction. The original magnification will be specified for each figure in the plates.

PLATE VI

All figures reduced five-twelfths.

FIG. 1.—*Verbena angustifolia* Michx.

FIG. 2.—Taxonomically intermediate form between *V. angustifolia* Michx. and *V. stricta* Vent.

FIG. 3.—*V. stricta* Vent.

FIGS. 4–6.—Taxonomically intermediate forms between *V. stricta* Vent. and *V. hastata* L.

FIG. 7.—*V. hastata* L.

FIGS. 8, 9.—Taxonomically intermediate forms between *V. hastata* L. and *V. angustifolia* Michx.

PLATE VII

FIGS. 18–25 magnified 700 diameters; fig. 26 magnified 800 diameters; figs. 22 and 25 are *V. hastata;* all the others *V. angustifolia.*

FIG. 18.—Details of ovule outlined in fig. 15, showing megaspore mother cell.

FIG. 19.—Nucellus of older ovule.

FIGS. 20, 21.—Megaspore mother cell nucleus dividing into two (20), and four (21).

FIG. 22.—Growth of fertile megaspore and its encroachment on sterile cells; nucellus cells somewhat stretched.

FIG. 23.—Embryo sac with 2 nuclei.

FIG. 24.—Embryo sac with 4 nuclei, reconstructed from 4 sections.

FIG. 25.—Embryo sac with polar nuclei in contact.

FIG. 26.—Details of a part of ovary outlined in text fig. 27, showing mature embryo sac invested by jacket; proteid-like substance in space between ovule and carpel.

PLATE VIII

FIGS. 28–41 magnified 700 diameters; figs. 42–45 magnified 800 diameters; figs. 42, 45 are *V. stricta;* all the others *V. angustifolia.*

FIG. 28.—Longitudinal section of young anther showing sporogenous cell row and surrounding layers.

FIGS. 29, 30.—Transverse and longitudinal sections through an older anther, showing granular and mostly binucleate tapetal cells: fig. 29, cells of middle layer, also granular; fig. 30, some rows of pollen mother cells, with nuclei in synapsis.

FIG. 31.—Three pollen mother cells in first division; tapetal cells with 2 nuclei.

FIG. 32.—Two pollen mother cells in anaphase of first division.

FIG. 33.—Early telophase of second division in pollen mother cell.

FIGS. 34, 35.—Tetrad formation; some cytoplasm of mother cell concerned in wall formation.

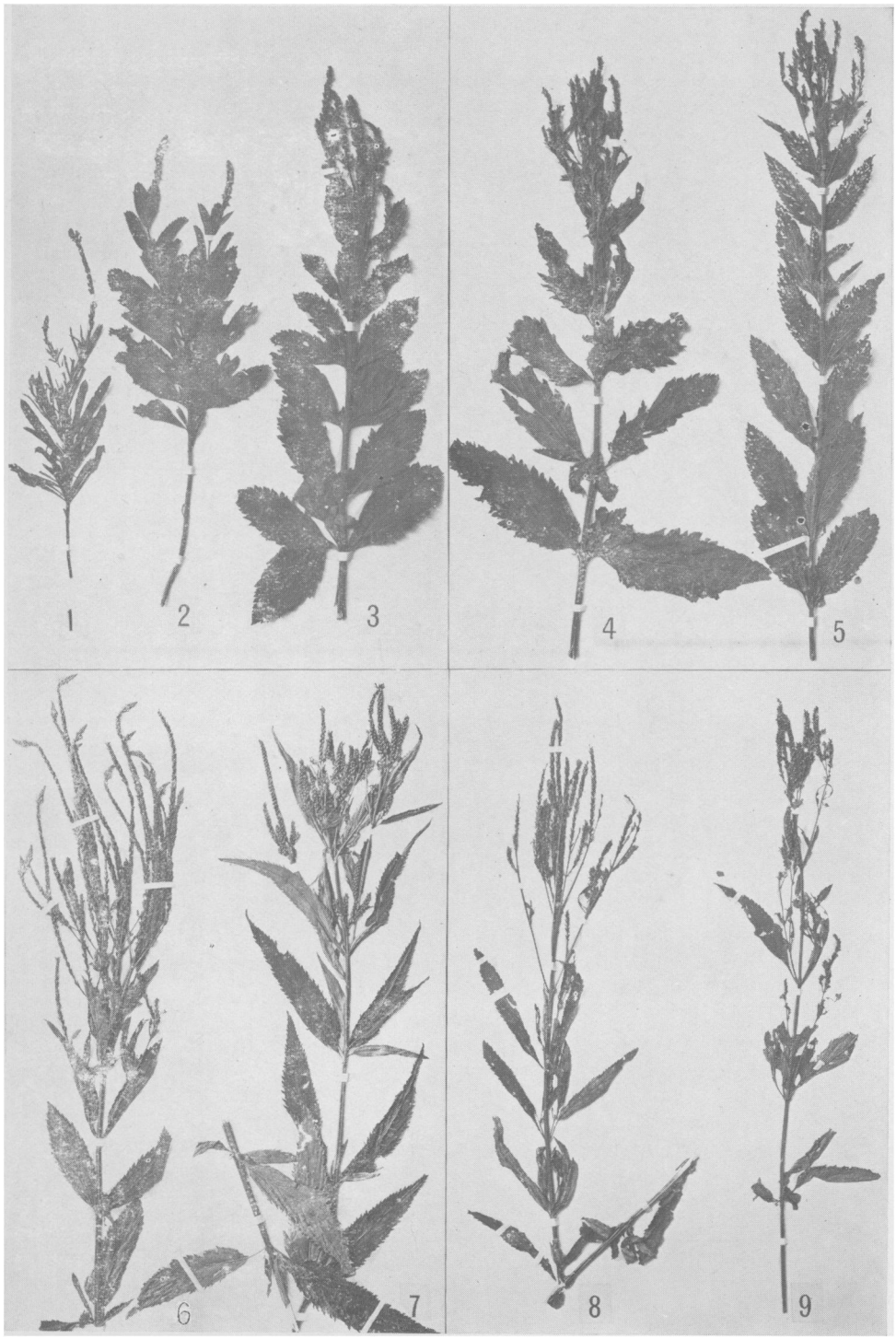
FIG. 36.—Tetrad formation; cytoplasm of mother cell not concerned in wall formation.

FIGS. 37–41.—Successive stages of development of pollen grain: fig. 39, pollen with starch grains; fig. 40, pollen with large vacuole; fig. 41, pollen with vegetative and generative nuclei.

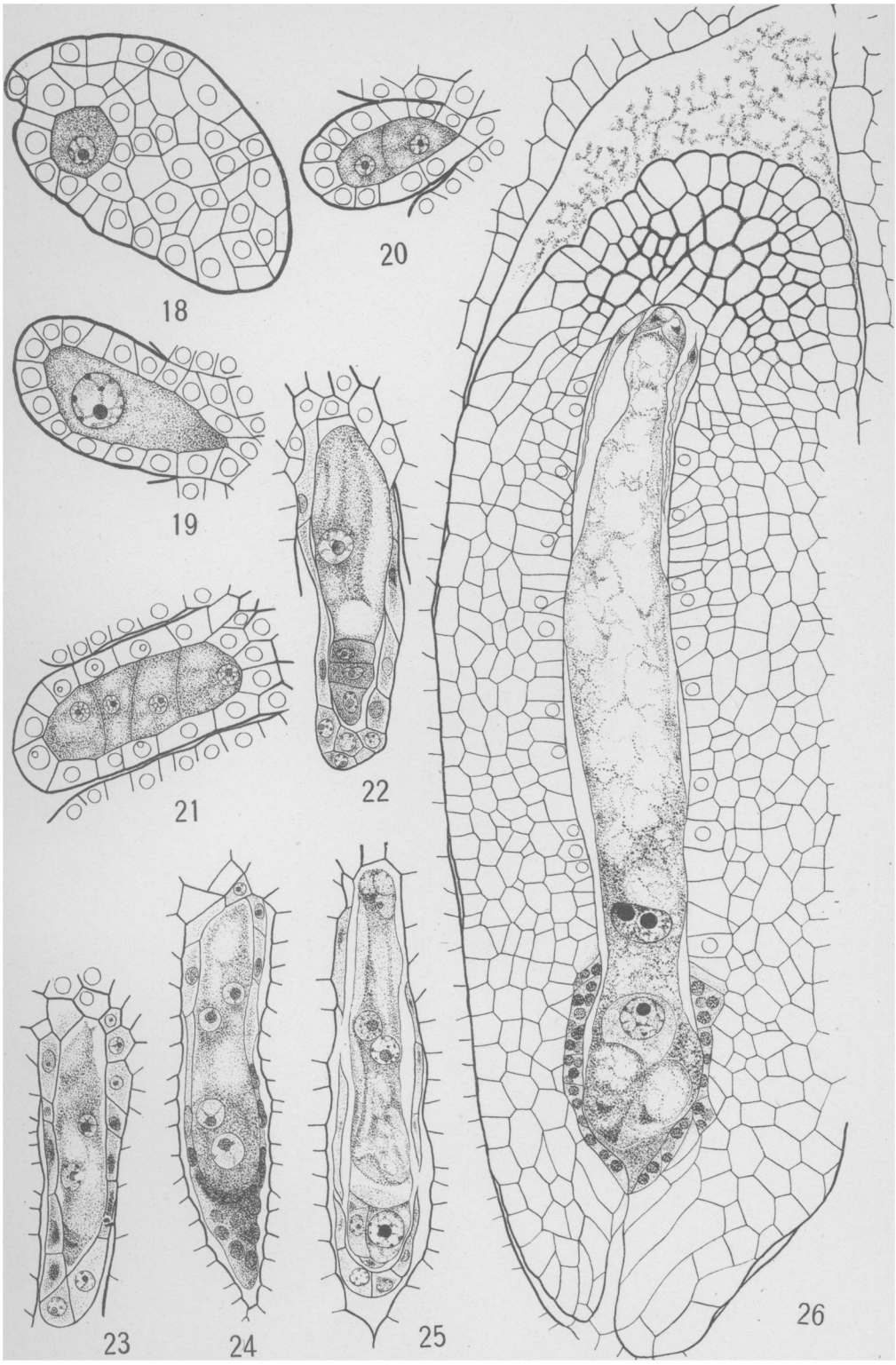
FIG. 42.—Pollen tube just thrusting itself through nucellar cap.

FIGS. 43, 44.—Fertilization: fig. 43, male nuclei fusing with egg and endosperm nucleus; pollen tube and starch grains shown.

FIG. 45.—First division of primary endosperm nucleus followed by wall formation.



KANDA on VERBENA



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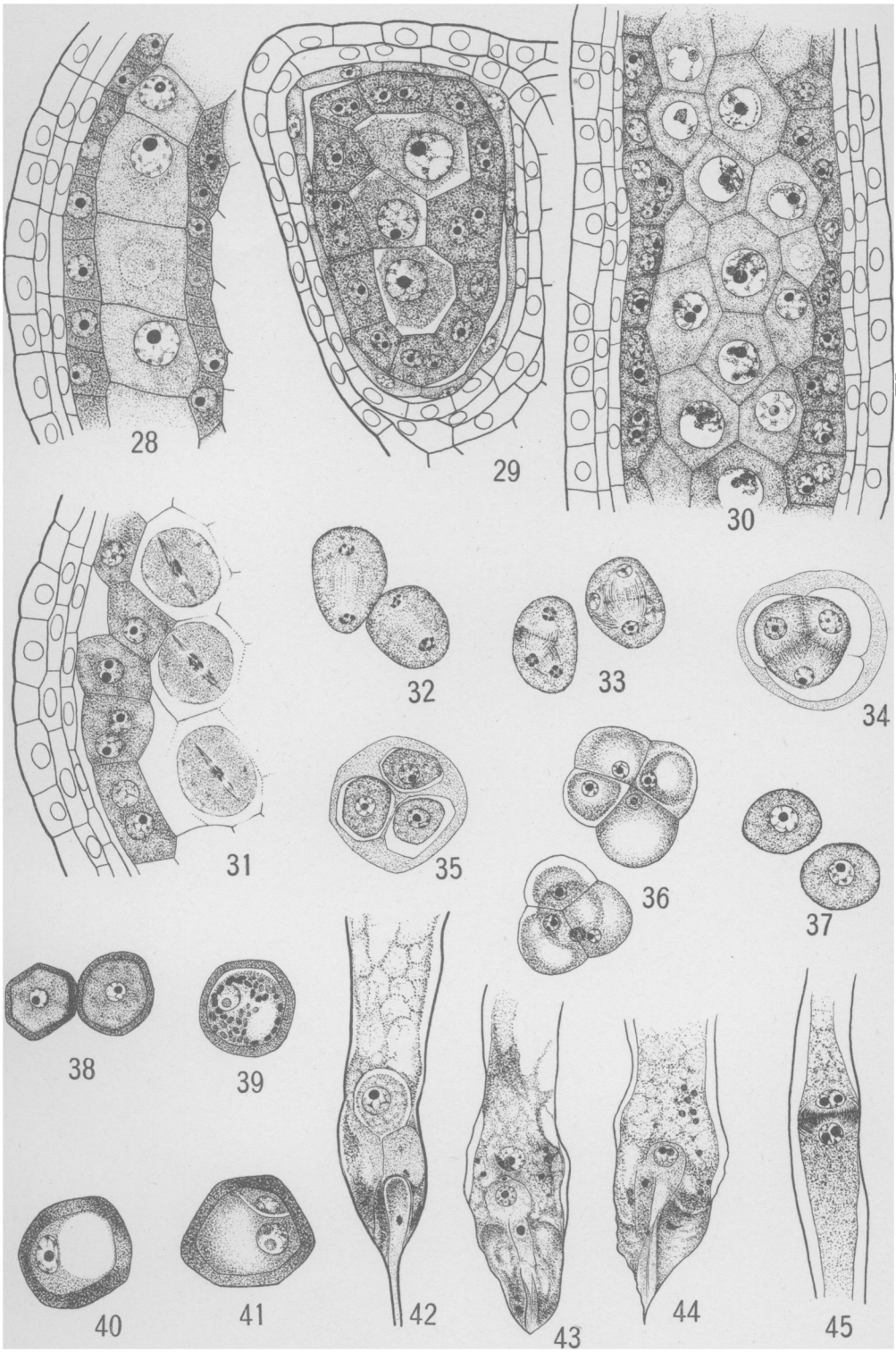

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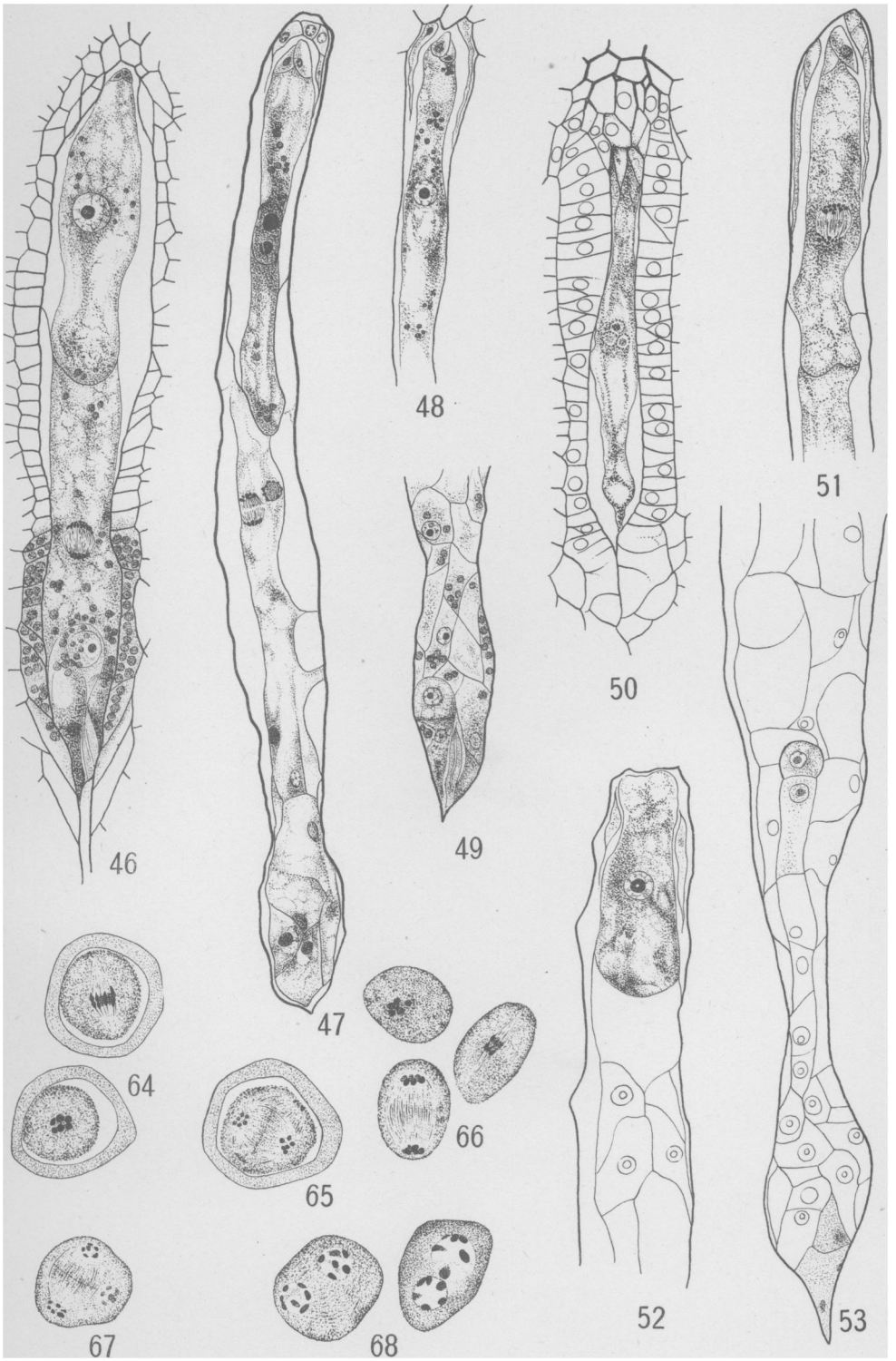

KANDA on VERBENA

PLATE IX

FIGS. 46–53 magnified 800 diameters; figs. 64–68 magnified 700 diameters; figs. 50, 68 are *V. hastata;* figs. 64, 65, *V. stricta;* figs. 66, 67, intermediate form between *V. stricta* and *V. hastata;* all others are *V. angustifolia.*

FIG. 46.—Embryo sac separated into micropylar and antipodal chambers: nucleus in micropylar chamber just in mitosis; reconstructed from 4 sections.

FIG. 47.—Embryo sac in which endosperm tissue is developing from micropylar end; single large undivided nucleus with 2 nucleoli in antipodal chamber.

FIGS. 48, 49.—Two portions of one embryo sac: fig. 48, antipodal chamber still 1-celled; fig. 49, micropylar chamber filled with tissue.

FIG. 50.—Embryo sac retarded in development by absence of starch in jacket; only 3 nuclei in center.

FIG. 51.—Mitosis of endosperm nucleus in antipodal chamber.

FIGS. 52, 53.—Two parts of more advanced embryo sac: fig. 52, antipodal part with one large resting cell; fig. 53, micropylar part with filamentous embryo.

FIGS. 64, 65.—Pollen mother cell in reduction division: fig. 64, metaphase of first division; fig. 65, early telophase of second division.

FIGS. 66, 67.—Pollen mother cells: fig. 66, metaphase and telophase of first division; fig. 67, telophase of second division.

FIG. 68.—Pollen mother cell in telophase of second division.